



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: HOFFMAN, Arnold et al. Examiner: James D.
Anderson

Application No.: 10/621,326 Art Unit: 1614

Filed: 4/17/2004

For: REDOX THERAPY FOR TUMORS

* * *

DECLARATION UNDER RULE 1.132

The Honorable Commissioner
of Patents and Trademarks
Box Non-Fee Amendment
Washington, DC 20231

Sir:

I, Arnold Hoffman, declare that:

1. I currently reside at 5 Hagra Street, Rehobot, Israel. I have personal knowledge of the facts set forth herein.
2. I am familiar with the Office Action dated 29 July 2009, which the Patent and Trademark Office mailed in regard to the above-captioned patent application. In response I declare and state as follows:
3. I previously submitted a Declaration under Rule 1.132 on June 18, 2009 averring to the contrasting results attained through prior art agents such as DSF and curcumin in vivo versus in vitro. In vitro results demonstrate that these agents are capable of effectively killing cancer cells, whereas in vivo results do not owing to an intrinsic in vivo impediment. Specifically, in vitro each agent inherently remains in continuous contact with the cancer cells for the duration of the treatment so that the dephosphorylated state of RB is continuously maintained. Consequently, the in vitro effectiveness of DSF by itself is at the maximum and there is no motivation to add or

combine any other agents.

4. The present application teaches that an effective Redox Therapy for treating cancer patients requires a treatment continuously maintaining the dephosphorylated state of RB from 15-75 hours. In contrast to in vitro, by its very nature, in vivo, agents do not inherently remain in continuous contact with the cancer cells for the duration of the treatment. Their absence allows the E to decrease before the next administration, resulting in phosphorylation of the RB, promoting cell proliferation. This explains why the prior art agents that may be very effective, in vitro, do not result in an in vivo therapy. The present inventor's solution was to combine different agents that deactivate the GR and/or the gamma-GCS enzymes that promote GSH formation, thereby maintaining a high E even after the agents are gone. Specifically, we use a combination of two E-increasing agents (disulfiram and curcumin) and two enzyme deactivating agents BCNU and BSO administered periodically, in a cumulative amount of from about 0.01-8 grams per day as needed to maintain a decreased $[GSH]^2/[GSSG]$ ratio in the malignant cells, thereby maintaining their dephosphorylated RB state over 15 to 75 hours (at least one cell cycle). This combination of four agents disclosed in the subject application provides in vivo synergy when administered in our prescribed dosage.

5. The Examiner has cited several prior art references that he claims make the subject application obvious. None of these cited references teach or suggest the combination of these agents, plus a pharmaceutically effective dosage comprising a calibrated administration frequency to continuously maintain the dephosphorylated state of RB (high E) for 15-75 hours.

6. One of the cited references is my own article Hoffman et al.(J Theor Biol, 2001, vol.211, pages 403-407). This paper teaches that raising E above

the threshold dephosphorylates the RB of the cells, halting cell proliferation which can lead to apoptosis. However, to prevent cell death of normal cells requires carefully increasing E by the administration of E-increasing agents so that only the E of the cancer cells is raised above the threshold. This is apparent from Fig 1 of my 2001 paper. This selectivity limits the 2001 therapy to direct administration of the agents into the tumor tissue, so that the E of the normal cells is not raised. My 2001 paper purports that any systemic administration of these agents will also increase the E of the normal cells, which according to the paper can lead to their apoptosis. Consequently, my 2001 paper teaches away from any systemic therapy by raising E, and one skilled in the art would need to look elsewhere for a systemic therapy.

7. In contrast, my present application teaches and claims a systemic therapy where raising the E of normal cells above the threshold does not harm them; it only harms cancer cells, and the application further explains why, paving the way for a safe systemic administration of the agents that increase E. At the time when the subject patent was filed, this was not obvious.

8. My prior 132 Declaration included results of our testing by systemic administration of the 4-agent combination (disulfiram, curcumin, BCNU and BSO) and demonstrated both safety and effectiveness.

9. We have since repeated systemic administration tests with varied amounts of this 4-agent combination. The Summary of the Protocol for this testing is attached hereto.

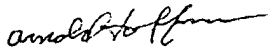
10. The Protocol Description of 002 and 012 are in vivo experiments and results of systemic administration of the four agents. Based on the cited prior art one skilled in the art would expect killing of both cancer cells and normal cells; i.e. toxicity. However, the

results clearly demonstrate a slowing down of tumor growth and no apparent toxicity. **In vitro** results also prove that the combination of 4 agents is very reproducible, and very effective over a longer period of time after washout. With respect to these parameters, the 4 agent combinations are better than 3 agent combinations, which, in turn are better than 2-agent combination, which, in turn, are better than single agent combinations. The halt in cell proliferation during treatment **observed in these experiments** reflects an increase in E that induced dephosphorylation of **RB** in accordance with the present claims.

* * * * *

I further declare that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or by both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the above referenced patent.

Signature:



Arnold Hoffman

Date: January 29, 2010

SUMMARY OF THE PROTOCOL

DSF, Curcumin, BSO and Carmustine Combination

ANTITUMOR ACTIVITY IN THE HUMAN BREAST CANCER XENOGRAPH (MX-1) MODEL IN MICE

Outline Protocol - Non GLP "Pilot" Study

*HBI Study No.:
HOF/012/EM*

*Submitted to:
Redoxia Ltd.*

*Attention:
Lee Spetner, Ph.D.*

*Prepared By:
Harlan Biotech Israel*

December 17, 2009

1. OBJECTIVE:

The objective of the study is to assess the antitumor activity of a combination treatment of 4 Test Items, comprising of *DSF (Tetraethylthiuram disulfide)*, *Curcumin*, *BSO (DL-Buthionine-sulfoximine)* and *Carmustine*, following once or twice, with 12 hours intervals, daily intravenous injections and oral administrations for a total of 4 or 7 administrations using MX-1, human mammary duct carcinoma, tumor model in female SCID-bg mice.

2. TEST MATERIALS:

(stock solution):	Clear, yellowish solution
Storage Conditions (crude material):	18-28°C
Storage Conditions (stock solution):	18-28°C
Expiry Date (crude material):	December 2010
Expiry Date (stock solution):	2 days following preparation

2.1.1 Curcumin:

Purchased From:	Sigma, Product No.: 28260
Manufacturer:	Fluka
Lot No.:	1386823
Characteristics & Physical State (crude material):	Orange powder
Characteristics & Physical State (suspension):	Orange suspension
Storage Conditions (crude material):	18-28°C
Storage Conditions (suspension):	18-28°C

2.1.2	Expiry Date (crude material):	March 2010
	Expiry Date (suspension):	2 days following preparation
	<u>BSO (DL-Buthionine-sulfoximine):</u>	
	Purchased From:	Sigma, Product No.: 19176
	Manufacturer:	Fluka
	Lot No.:	1333005
	Characteristics & Physical State (crude material):	White powder to fine crystals
	Characteristics & Physical State (solution):	Clear, colorless solution
	Storage Conditions (crude material):	2-8°C
	Storage Conditions (stock solution):	2-8°C
	Expiry Date (crude material):	December 2011
	Expiry Date (stock solution):	2 days following preparation
2.1.3	<u>Carmustine:</u>	
	Purchased From:	Sigma, Product No.: C0400
	Lot No.:	038K4008 or 039K4098
	Characteristics & Physical State (crude material):	Light yellow powder
	Characteristics & Physical State (solution):	Clear light yellow solution
	Storage Conditions (crude material):	(-18)-(-22)°C and protected from light
	Storage Conditions (stock solution):	18-28°C and protected from light
	Expiry Date (crude material):	January 2010 or December 2010
	Expiry Date (stock solution):	2 days following preparation

2.2 Adjunct Items:

2.2.1	<u>Water For Injection (WFI):</u>	
	Lot No.:	8451-02
	Manufactured by:	Norbrook Laboratories Ltd.
	Characteristics and Physical State:	Clear, colorless solution
	Storage conditions:	18-28°C until first use, 2-8°C thereafter
	Expiry Date:	November 2010
2.2.2	<u>DMSO (Dimethyl Sulfoxide):</u>	
	Batch No.:	078K0692
	Purchased From:	Sigma, Product No.: D5879
	Characteristics and Physical State:	Clear, colorless solution
	Storage conditions:	18-28°C
	Expiry Date:	August 2010
2.2.3	<u>Ethanol Absolute:</u>	
	Batch No.:	L90002359
	Purchased From:	Gadot Laboratory Supplies Ltd.
	Characteristics and Physical State:	Clear, colorless solution
	Storage conditions:	18-28°C

Expiry Date:

July 2012

3. TEST SYSTEM:

- 3.1 Test Animals: Mouse / C.B-17/IcrHsd-Prkdc^{scid}Lyst^{bg}
- 3.2 Sex: Female
- 3.3 Group Size: $n=6$ at most
- 3.4 Animal Husbandry: (i) Acclimatization of at least 5 days.
- (ii) All test animals are kept under environmental controlled housing conditions throughout the entire study period, and maintained in accordance with Harlan Biotech Israel (HBI) approved Standard Operation Procedures (SOP's).

4. CONSTITUTION OF TEST GROUP AND DOSE VOLUME:

Group No.	Group Size	T R E A T M E N T				
		Experimental Material	Route of Administration	Frequency & Duration	Dose Level (mg/kg)	Volume Dosage (ml/kg)
1F	$n=2$	Curcumin	PO	BID \times 3.5d	250	10
		BSO & Carmustine Combination	IV		500 & 0.04 respectively	
		DSF	PO		20	
2F	$n=2$	Curcumin	PO	BID \times 3.5d	250	10
		BSO & Carmustine Combination	IV		500 & 0.4 respectively	
		DSF	PO		20	

5. MODEL INDUCTION:

The Mammary Xenograft-1 (MX-1), a human derived mammary duct carcinoma, fragments employed in the current study were derived from untreated animals (from previous HBI studies - HOF/006,009/EM) and kept frozen (in liquid nitrogen) until the time of implantation. Tumor fragments for the conduct of the main phase are freshly prepared, from tumors grown in mice of the preliminary phase, by HBI according to the NCI recommended transplantation protocol.

5.1 Tumor Implantation Procedure:

To obtain the required amount of animals for the study the procedure is performed in 2 steps:

1. The frozen fragments are implanted immediately after thawing. Each vial containing fragments, is rapidly thawed (~120 seconds) in a hot water bath at 37-40 °C. Incision is made at the right lateral midline dorsal area of 3 naive mice and a pocket is created at the right sub-axillary area by gentle blunt dissection. Then two fragments (approximately 2×2×2 mm each) are implanted subcutaneously close to each other at the created pocket.
2. Following tumor mass growth, the donor animals are euthanized, the tumors are excised, dissected and transferred to a sterile petri dish placed over ice and cut into approximately 2×2×2 mm fragments. Then, incision is made at the right lateral midline dorsal area of 16 naive mice and one fragment is implanted subcutaneously into the right flank area of each animal.

5.2 Tumor Monitoring (Pre-Treatment):

Tumor growth is monitored following implantation until visually measurable tumors are observed and thereafter at least twice weekly (not on Saturdays), by calculating the total subcutaneous mass (mm^3) determined on the basis of width and length measurements (as detailed in Subsection 6.1) using digital calipers. As specified by the Sponsor, treatment commencement (Day 0), is to be carried out on either Sunday, Monday or Tuesday morning, to prevent any dosing on Saturday, after tumors develop a mean volume of approximately $110\text{-}170\text{ mm}^3$ (~6-7 mm in diameter).

Note: Tumor volume of $110\text{-}170\text{ mm}^3$ represent the standard volume for study commencement; however the Sponsor specific requirement is that treatment is to be initiated on either Sunday, Monday or Tuesday morning therefore treatment maybe initiated in animals with tumor size, which is out of the standard range.

5.3 Body Weight (Pre-Treatment):

Individual body weights are determined just prior to MX-1 fragment transplantation, 2 days following transplantation and twice weekly thereafter until initiation of treatment.

5.4 Treatment Initiation, Frequency and Duration:

As requested by the Sponsor, the daily injections are performed in successive days unless a loss of more than 10% of the initial body weight (prior to first dosing session) is observed in any individual animal. In this case, dosing is ceased for that animal until the animal has regained its weight up to at least 90% of its initial body weight, where at this point dosing is continued (but no more than a week interval between administrations). This regimen is to be repeated until all dosing sessions are completed.

5.4.1 Groups 1F-2F:

Upon having at most $n=4$ mice bearing each a tumor of approximately $110\text{-}170\text{ mm}^3$ (taking into consideration the "Note" above), treatment is initiated by twice daily administrations (on weekdays with inter-dosing intervals of about 12 hours) for a period of 3.5 days, to a total of 7 administrations.

5.5 Route of Administration, Dose levels and Volume Dosage:

5.5.1 Oral Route:

Due to the fact that Test Items are prepared with 2 different solvents, which we don't know how they react together, oral administration by the use of a suitable stainless steel feeding needle is performed as follows: Curcumin at 250 mg/kg is administered following by DSF at 20 mg/kg (about half an hour between dosings) to all treated groups (Groups 1F-3F). Both Test Items administered at a volume dosage of 10 ml/kg .

5.5.2 Intravenous Route:

BSO at dose level of 500 mg/kg and Carmustine at dose levels of 0.04 or 0.4 mg/kg combination are administered to groups 1F or 2F & 3F, respectively, by intravenous (IV) injection into one of the tail veins, using a suitable syringe and needle, at a volume dosage of 10 ml/kg . In case IV injection cannot be

performed, due to the multiple injections, the Test Items combinations are injected intraperitoneally (IP) into either the left or right caudal abdominal

OBSERVATIONS AND EXAMINATIONS:

Observations and examinations are to be carried out throughout the treatment period and up to a total of 2 weeks. If dosing is to be delayed, as detailed in subsection 5.4.1, the total duration of the observation period is to be determined following consultation with the Sponsor.

5.6 Tumor Growth Monitoring:

Monitoring of progressive changes in tumor growth is carried out prior to treatment, and ~~twice daily~~ thereafter (excluding Saturday) until study termination.

The equation used for tumor volume determinations is:

$$V (mm^3) = d^2 (mm^2) \times D (mm) / 2$$

Where: d and D are represent the smallest and the largest perpendicular tumor diameters respectively.

5.7 General Observation:

Animals are observed at least once daily during regular working days for signs of morbidity, mortality and any other unusual reactions with special emphasis on the tumor development area.

5.8 Body Weight:

Individual body weights are determined prior to first dosing session and once daily thereafter until study termination.

5.9 Study Termination:

At the end of the study animals are euthanized by CO₂ asphyxiation tumors are excised and weighed.